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(54) Title: LYMPHOCYTE ACTIVATION ANTIGEN HB15, A MEMBER OF THE IMMUNOGLOBULIN SUPERFAMILY

(57) Abstract

Lymphocyte activation antigen HB15, and the human cDNA and gene sequences encoding HB15, are disclosed. HB15 is not expressed at detectable levels by circulating leukocytes but has a unique pattern of expression among tissues. HB15 is uniquely expressed by Langerhan's cells within the skin and other subpopulations of dendritic cells. Also disclosed are antibodies reactive with HB15 and methods of using anti-HB15 antibodies, or other antagonists to HB15 function, to treat an immunological disorder, disease or syndrome.

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LYMPHOCYTE ACTIVATION ANTIGEN HB15, A MEMBER OF THE IMMUNOGLOBULIN SUPERFAMILY

FIELD OF THE INVENTION

This invention relates to nucleic acid sequences encoding human lymphocyte activation antigens, particularly to sequences encoding lymphocyte activation antigen HB15, and to the proteins and polypeptides encoded by those sequences.

Part of the work leading to this invention was made with United States Government funds. Therefore, the U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Many of the cell-surface molecules which regulate immune responses contain conserved structural features similar to those found in immunoglobulin (Ig). These molecules are encoded by genes that are presumed to have evolved from a common precursor and are therefore members of a large superfamily (Williams et al., Annu. Rev. Immunol., 88:381-405 (1988)). Many of the Ig superfamily members are involved in cell-cell adhesion and signal transduction. While most members of this family contain multiple linearly-assembled Ig-like domains, several proteins have been identified that contain single Ig-like domains. Single Ig-like domain proteins that are known or assumed to be involved in cell-cell adhesion include: CD8α (Littman et al., Cell 40:237 (1985)), CD8ß (Johnson et al., Nature 323:74 (1986)), CD7 (Aruffo et al., EMBO J. 6:3313 (1987)), Thy-1 (Williams et al., Science 216:696 (1982)), CD28 (Aruffo et al., Proc. Natl. Acad. Sci. USA 84:8573 (1987)), CTLA-4 (Brunet et al., Nature 328:267 (1987)) and Po which is a structural protein of the peripheral myelin sheath (Lemke et al., Cell 40:501 (1985)). In addition, others associate with the antigen receptors of B and T lymphocytes forming multimeric signal-transducing complexes including; CD3 γ, δ and ϵ chains (Gold

et al., Nature 321:431-434 (1986); van den Elsen et al., Nature 312:413-418 (1984)), B29 (Hermanson et al., Proc. Natl. Acad. Sci., USA 85:6890 (1988)), and mB1 (Sakaguchi et al., EMBO J. 7:3457-3464 (1988)).

Two single Ig-like domain containing proteins found on lymphocytes are preferentially associated with cellular activation and are known to be involved in mediating cell-cell interactions. CD28 is expressed much more on activated than nonactivated T and B lymphocytes (Turka et al., J. Immunol. 144:1646 (1990)), and CTLA-4 is expressed mostly, if not exclusively, by activated T and B lymphocytes (Brunet et al., Nature 328:267 (1987); Harper et al., J. Immunol. 147:1037-1044 (1991)). The role of CD28 as a T cell receptor for the B7 molecule expressed by activated B cells has been recently identified (Linsley et al., Proc. Natl. Acad. Sci. USA 87:5031-503 (1990); Freeman et al., J. Immunol. 143:2714-2722 (1989)), as has a similar role for CTLA-4 (Linsley et al., J. Exp. Med. 174:561-569 (1991)). As with CD28 and B7, most of the Ig-like domain-containing receptors interact with other members of the Ig superfamily present on other cells.

SUMMARY OF THE INVENTION

cDNA cloned from a human lymphocyte library were analyzed and shown to encode a novel cell-surface glycoprotein, termed HB15, expressed by activated lymphocytes. The mature 186 amino acid protein encoded by the cDNA was composed of a single extracellular V type immunoglobulin (Ig)-like domain, a transmembrane domain and a 39 amino acid cytoplasmic domain. Northern blot analysis revealed that HB15 derives from three mRNA transcripts of ~1.7, 2.0 and 2.5 kb expressed by lymphoblastoid cell lines. Monoclonal antibodies reactive with HB15 were produced and used to show that HB15 is expressed as a single chain cell-surface glycoprotein of M_r 45,000. HB15 expression was specific for lymphoblastoid cell lines and mitogen-activated lymphocytes; HB15 was not expressed at detectable levels by

circulating leukocytes. Immunohistological analysis revealed that HB15 has a unique pattern of expression among tissues, being found predominantly in hematopoietic tissues with scattered expression by interfollicular cells and weak expression by mantle zone and germinal center cells. Uniquely, HB15 is also expressed by Langerhan's cells within the skin and circulating dendritic cells. Thus, the HB15 glycoprotein represents a new member of the Ig superfamily.

cDNA sequences encoding the HB15 protein or portions thereof, including any of its specific domains, ligand binding fragments or immunospecific fragments, can be incorporated into replicable expression vectors and the vectors transfected into an appropriate host (e.g., a bacterial, yeast, or eucaryotic cell culture). Alternatively, genomic DNA fragments encoding the HB15 protein or portions thereof can be utilized *in situ*. The expressed proteins or polypeptides, or antagonists thereto, can be used to modulate mammalian immune function. Also, the expressed products can be employed as immunogens in order to raise antibodies against HB15 or portions thereof including any of its specific domains or fragments thereof.

Thus, the invention generally features nucleic acid isolates encoding lymphocyte activation antigen, HB15, or portions thereof including any of its specific domains, ligand binding fragments or immunospecific fragments; the encoded HB15 protein or portions thereof including specific domains, ligand binding fragments and immunospecific fragments; methods of producing HB15 or portions thereof; methods of detecting the presence of HB15 or of an HB15 ligand; methods of identifying or developing antagonists to HB15 or HB15 ligand function; methods of diagnosing or treating a patient suffering from an immunological disorder, methods of identifying or of isolating cells that express HB15 or fragments thereof, and antibodies reactive with HB15 or fragments thereof.

Also featured are derivatives of HB15 having variant amino acid sequences or glycosylation not otherwise found in nature, the nucleic acid isolates encoding such derivatives, and polynucleotide probes capable of hybridizing under stringent conditions to the HB15 gene.

As used herein the term "antagonist to HB15" includes any agent which interacts with HB15 and interferes with its function, e.g., antibody reactive with HB15 or any ligand which binds to HB15. The term "identify" is intended to include other activities that require identification of an entity, such as isolation or purification. The terms "isolated" or "essentially purified" refer to a nucleic acid or protein sequence that has been separated or isolated from the environment in which it was prepared or in which it naturally occurs. Such nucleic acid or protein sequences may be in the form of chimeric hybrids, useful for combining the function of the nucleic acid or protein sequences of the invention with other species. The term "immunospecific fragment" refers to a fragment of the indicated protein that reacts with antibodies specific for a determinant of the indicated protein.

The HB15 protein, immunospecific or ligand binding fragments or specific domains thereof, or other antagonists to HB15 that interfere with HB15 function, can be used therapeutically to modify or inhibit the development or progression of an immune response or cellular interaction, or to deliver drugs, toxins, or imaging agents to cells that express HB15. HB15 cDNA can be used to produce these proteins or peptide fragments; to identify nucleic acid molecules encoding related proteins or polypeptides (e.g., homologous polypeptides from related animal species and heterologous molecules from the same species); or to build other new, chimeric molecules having similar function either in transformed cells or in cell free systems. In addition, HB15 cDNA can be used to synthesize antisense

oligonucleotides for inhibiting the expression of the HB15 protein. Assays for HB15 function, production or expression by cells are made possible by the development of monoclonal antibodies selectively reactive with the HB15 protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the structure of the HB15 cDNA clone and the location of restriction sites;

Fig. 2 shows the cDNA nucleotide sequence and the deduced amino acid sequence of HB15;

Fig. 3 shows a hypothetical model for the structure of the extracellular domain of HB15;

Figs. 4A and 4B show the immunofluorescence results obtained with three lymphoblastoid cell lines that express HB15; and

Figs. 5A-5F show immunohistochemical analysis of HB15 expression.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The lymphocyte activation antigen, HB15, is expressed exclusively by lymphoid tissue and skin Langerhan's cells. Referring to Fig. 1, the structural features of the HB15 protein, predicted from nucleotide sequence derived from multiple cDNA clones, clearly establish it as a new member of the Ig superfamily. The predicted structure of HB15 is that of a typical membrane glycoprotein with a single extracellular Ig-like domain, a transmembrane domain and an approximately 40 amino acid cytoplasmic domain. It is likely that the entire coding region for HB15 was identified as transfection of cell lines with the pHB15 cDNA generated cell surface expression of the protein and the M_r of the immunoprecipitated protein was similar in both cDNA transfected cells (~45,000) and HB15$^+$ Raji cells (~40,000).

It is also likely that HB15 undergoes extensive post-translational processing as HB15 was expressed as a single chain molecule, yet the determined M_r was twice the predicted size of the core protein. Since HB15 was also expressed on the surface of cDNA transfected cells, including COS cells, CHO cells, a mouse pre-B cell line and a human erythroleukemia line, it is likely that surface expression is not dependent on expression of other components of a molecular complex as occurs with the Ig-like proteins that associate with the T and B cell antigen receptors.

Comparison of the HB15 amino acid sequences with other previously identified proteins did not reveal any striking homologies, except the similarity of the extracellular Ig-like domain with other members of the Ig superfamily. The HB15 Ig-like domain contained many of the conserved features found in the V-set of domains as shown in Fig. 2 (Williams et al., Ann. Rev. Immunol. 88:381-405 (1988)). Based on the homology with Ig domains, HB15 is likely to possess a disulfide bond linking Cys 16 and Cys 88. This would place 71 amino acids between the two Cys residues which is of the appropriate size for V-related domains (Williams et al., supra). There is the potential for additional disulfide bond formation between residues at positions 8, 81 and 110 since these Cys are present in the extracellular domain as well. In addition, HB15 has a Cys residue located within the predicted membrane spanning domain at position 144. Cys residues are also located at identical positions in CD3δ and CD7, suggesting some functional significance, perhaps as sites for fatty acylation (Kaufman et al., J. Biol. Chem. 259:7230-7238, (1984); Rose et al., Proc. Natl. Acad. Sci., USA 81:2050-2054 (1984)). The HB15 cytoplasmic tail is similar in size to that of CD7 (Aruffo et al., EMBO J. 6:3313 (1987)), but shared no amino acid sequence similarity with known proteins. However, the five Ser/Thr residues within this domain could serve as potential sites of phosphorylation. Thus, HB15 appears to be a newly described

lymphocyte cell surface antigen that shares no apparent relatedness with previously described structures.

The HB15 extracellular domain is different from the typical Ig-like domain in that it is encoded by at least two exons. Analysis of partial genomic DNA sequence revealed that half of the Ig-like domain is encoded by a single exon and the putative membrane spanning domain is also encoded by a distinct exon (Fig. 2). That Ig-like domains can be encoded by more than one exon has been observed for some members of the Ig superfamily, including the Po protein (Lemke et al., Neuron 1:73-83 (1988)), CD4 (Littman et al., Nature 325:453-455 (1987)) and N-CAM (Owens et al., Proc. Natl. Acad. Sci., USA 84:294-298 (1987)). This finding supports structural analyses which suggested that Ig domains may have arisen from an ancestral half-domain that evolved through duplication and subsequent adjoining. However, each of the above genes and the HB15 gene contain introns at different locations between the sequences coding for the conserved Cys residues of the disulfide bond (Williams et al., Annu. Rev. Immunol. 88:381-405 (1988)). This finding supports the notion that introns may have been subsequently inserted to interrupt the ancestral Ig-like domain at later points during the evolution of each of these domains.

Expression of HB15 appears to be generally restricted to lymphocytes since two monoclonal antibodies reactive with HB15 failed to detect HB15 on most other hematopoietic cells. HB15 expression may be a late event in lymphocyte development as most thymocytes and circulating lymphocytes did not express detectable levels of cell surface HB15. However, after being activated by mitogens, peripheral lymphocytes expressed maximal levels of cell surface HB15 on days 3 through 5, the period of time during which maximal proliferation occurred. HB15 may be expressed at low levels by monocytes, especially after culture or activation, but the level of expression is low and may just result from Fc receptor mediated antibody attachment. Many T and B cell

lines also expressed HB15, but expression was generally at low levels. Interestingly, cell-surface HB15 expression by cell lines was highest during periods of maximal proliferation such as on the first day after the cultures were fed. These results imply that HB15 is important for maximal growth of lymphoblastoid cells or the maximal growth of cells is critical for the expression of this antigen. This was consistent with the observation that HB15 was expressed by germinal center cells in hematopoietic tissues. Nevertheless, HB15 expression appeared to be lymphoid tissue restricted as revealed by immunohistological analysis of twenty-two different tissues. The only exception was the finding that skin Langerhan's cells express HB15. This unique pattern of restricted expression, along with the structural analysis of the protein, indicates that HB15 is a newly identified lymphocyte activation antigen.

The structural similarity of HB15 with other members of the Ig superfamily suggests that it may be involved in cellular interactions since Ig-like domains are frequently involved in a variety of homotypic and heterotypic interactions in the immune and nervous systems. These interactions include binding functions that trigger a subsequent event below the cell surface or adhesion. A key functional feature is that homophilic or heterophilic binding usually occurs between Ig-related molecules, and this is often between molecules on opposed membrane surfaces. The structural relatedness of HB15 to these other proteins may imply a role for this lymphocyte activation protein in either homotypic or heterotypic interactions of lymphocytes following activation or other $HB15^+$ cell types.

It is understood that the particular nucleotide and amino acid sequences disclosed in Fig. 2 are representative of the counterpart and related human genes and proteins that can conveniently and directly be obtained following the teaching of this disclosure. For example, cross-hybridization under stringent conditions of the disclosed

nucleic acid sequences with genetic material from human cells, can readily be performed to obtain equivalent human sequences. In an analogous manner, degenerate oligonucleotides can readily be synthesized from the disclosed amino acid sequence, or portions thereof, and amplified using any well-known amplification technique, such as the polymerase chain reaction, to obtain probes that bind to equivalent human sequences. Proteins or polypeptides encoded by equivalent sequences can be produced. Antibodies directed against the disclosed protein or peptides can also be raised and employed to cross-react with human and other mammalian peptides having similar epitope(s). Those peptides isolated in this manner that have similar antibody reactivity patterns to those of the disclosed proteins or peptides are considered equivalents of the disclosed proteins or peptides.

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

EXAMPLE I

Isolation and characterization of HB15 cDNA clones and characterization of the HB15 protein

A human tonsil cDNA library was screened by differential hybridization using labeled cDNA from the B lymphoblastoid cell line Raji and the T cell line H-SB2. Two of the 261 $RAJI^+$ $H\text{-}SB2^-$ cDNA clones isolated, pB10 (~2.5 kb) and pB123 (~1.2 kb), cross hybridized, yet failed to hybridize with cDNA that encode known B cell surface antigens (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)). Expression of this mRNA was examined by Northern blot analysis using poly$(A)^+$ RNA isolated from B cell lines (NALM-6, Namalwa, Daudi, SB, and Raji), T cell lines (Hut-78, H-SB2, and MOLT-3) and the erythroleukemia line, K562. The pB123 cDNA hybridized strongly with three mRNA species of

~1.7, ~2.0 and ~2.5 kb in SB and Raji. Daudi and Namalwa cells expressed lower levels of this mRNA. Further autoradiography of the blot (7 days) revealed that the NALM-6, Hut-78 and MOLT-3 cells also expressed these three mRNA species, but at much lower levels, and faint hybridization with H-SB2 RNA was detected. These results suggested differential expression of this gene among leukocyte subpopulations.

Restriction maps were generated for these cDNA and their nucleotide sequences determined. Both cDNA were overlapping and contained open reading frames at their 5′ ends with the pB123 cDNA having the longest 5′ sequence. Since neither clone contained a translation initiation site, the pB10 cDNA insert was used to isolate 13 additional cross-hybridizing cDNA from a human tonsil library. Restriction maps and nucleotide sequence determination indicated that 12 of the cDNA were overlapping, with one cDNA having the longest sequence at the 5′ end. The restriction map and nucleotide sequence of this clone, termed pHB15, is shown in Fig. 1. The full length cDNA clone is likely to include an ~500 bp fragment at the 3′ end that was removed from the cDNA by EcoR I digestion and subcloning. Eight other independent cDNA clones had similar EcoR I generated fragments and an EcoR I site was located at the identical nucleotide position in all cDNA that were sequenced.

The pHB15 cDNA had a 625 bp open reading frame, with the major portion of the cDNA representing untranslated sequence. The determined nucleotide sequence and predicted amino acid sequence of HB15 are given in Fig. 2. The predicted cleavage site used to generate the mature protein is shown by a vertical arrow. The numbers shown above the amino acid sequence designate amino acid residue positions of the putative mature protein and the numbers on the right designate nucleotide positions. Amino acids are designated by the single-letter code, and * indicates the termination codon. Nucleotides delineating translated regions with

hydrophobic character are underlined. Amino acids indicating potential N-linked glycosylation attachment sites are underlined. A poly(A) attachment signal sequence is indicated by wavy underlining. The Cys residues are circled and amino acids which are often conserved in Ig-like domains are indicated by (+). Arrow heads below the nucleotide sequence denote exon/intron boundaries identified in another DNA clone.

The first ATG shown is the most likely initiation codon for translation since it conforms to the proposed translation initiation consensus sequence, (A/G)CCAUG (Kozak, Cell 44:283-292 (1986)). It is likely that the different mRNA species result from differential use of poly(A) attachment sites, AATAAA, since one was found at nucleotide position 1248 in the middle of the 3′ untranslated region (Fig. 2). This poly(A) attachment site was functional in the pB123 cDNA since it was followed by a poly(A) tail. A poly(A) attachment site or tail was not found in the ~550 bp EcoR I fragment which presumably represents the 3′ end of the pHB15 cDNA.

One clone isolated from the cDNA library (~3.0 kb long) that hybridized with the pB123 cDNA had a unique sequence with 229 and 107 bp long segments that were identical to those found in the other cDNA. These regions had flanking sequences that corresponded to the consensus 5′ and 3′ splice sequences which demark exon boundaries (Aebi et al., Trends Genet. 3:102-107 (1987)) indicating that this aberrant cDNA was composed of introns and two exons. The three splice junction sites identified by this clone are shown (Fig. 2).

The predicted length of the HB15 protein was 205 amino acids (Fig. 2). However, the pB123 cDNA was missing the codon AAG at nucleotide position 500 so the protein may be one amino acid shorter in some cases. This may result from differential splicing at an exon/intron border, that results in the inclusion or loss of a codon since this codon abuts a potential splice site. A similar phenomenon has been found

in the CD19 gene which also encodes a member of the Ig superfamily (Zhou et al., Immunogenetics 35:102-111 (1992)). Hydropathy analysis of the HB15 amino acid sequence by the method of Kyte et al., J. Mol. Biol. 157:105 (1982) revealed two regions of strong hydrophobicity. The first hydrophobic stretch of 19 amino acids represents a typical signal peptide at the amino terminal end of the protein. The algorithm of von Heijne, Nucleic Acids Res. 14:4683-4690 (1986) predicts that the most probable amino-terminus of the mature protein would be the Thr following amino acid 19. The second hydrophobic region of 22 amino acids most probably represents the transmembrane region. Three potential N-linked glycosylation attachment sites (N-X-S/T) were found in the extracellular domain. Therefore, the predicted molecular mass of the core protein would be ~20,500.

Six Cys residues were found in the extracellular domain of HB15 and one in the putative membrane spanning domain. One pair of these residues at positions 16 and 88 delineate Ig-like domains (Williams et al., Annu. Rev. Immunol. 88:381-405 (1988)). This domain contained many of the hallmark amino acids which define the V set of Ig-like domains. A computer search of protein sequences using the Protein Identification Resource Protein Sequence Database showed that no proteins shared significant sequence homology with HB15 other than some members of the Ig superfamily.

Referring to Fig. 3, a hypothetical model is given for the structure of the extracellular domain of HB15 based on the proposed arrangement of the ß-pleated sheets for the V domain of Ig heavy chain. Cys residues are represented as filled circles and amino acids encoded by different exons are indicated by alternatively shaded circles. Numbers represent the predicted amino acid residue positions as in Fig. 2.

EXAMPLE II

Production of monoclonal antibodies reactive with HB15.

Hybridomas were generated by the fusion of NS-1 myeloma

cells with spleen cells obtained from mice immunized with pHB15 cDNA-transfected COS cells. Monoclonal antibodies reactive in indirect immunofluorescence assays with HB15 mRNA positive cell lines, but not with HB15 negative cell lines, were isolated. Two of these antibodies, anti-HB15a (IgG_{2b}) and anti-HB15b (IgG_3) also reacted with COS cells transfected with the pHB15 cDNA, but did not react with cells transfected with CD19 cDNA (Tedder et al., J. Immunol. 143:712-717 (1989)) or the expression vector alone. In addition, these antibodies reacted with a human erythroleukemia cell line, K562, and a mouse pre-B cell line, 300.19, stably transfected with the pHB15 cDNA. The antibodies did not react with untransfected parent cells, cells transfected with vector alone; or CD19, CD20 (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)) or LAM-1 (Tedder et al., J. Exp. Med. 170:123-133 (1989)) cDNA transfected cells. In all cases, the reactivities of the anti-HB15a and anti-HB15b mAb were identical.

EXAMPLE III

Detection of HB15 expression.

Immunoprecipitation of cell surface HB15.

The anti-HB15a mAb was purified, coupled to beads and used to immunoprecipitate HB15 from detergent solubilized extracts of surface-iodinated cell lines. Optimum results were obtained using the K562-HB15 cell line (K562 cells transfected with pHB15 cDNA) since the level of HB15 expression was higher than in other cell lines. The anti-HB15a mAb specifically immunoprecipitated proteins that migrated as a single broad band of ~45,000 M_r. Similar results were obtained when the immunoprecipitated materials were run under reducing or nonreducing conditions. A similar protein was immunoprecipitated from the Raji cell line except the M_r was ~40,000. Thus, HB15 was expressed as a noncovalently-associated single chain molecule on the cell surface.

HB15 was expressed by activated lymphocytes.

The tissue distribution of the HB15 surface antigen was examined by indirect immunofluorescence staining with flow cytometry analysis. Two cell lines that did not express HB15 message were transfected with the pHB15 cDNA subcloned into the Bam HI site of the retroviral vector pZipNeoSV(X). Referring to Fig. 4, the immunofluorescence results obtained with three lymphoblastoid cell lines that express HB15 are demonstrated. The open histograms show the cellular reactivity with the HB15a antibody, and the shaded histograms demonstrate background levels of immunofluorescence staining obtained with unreactive control antibodies. Among 33 cell lines examined, HB15 was expressed at detectable levels by B cell lines (including Raji, Daudi, Namalwa, Arent, BJAB, SB, Jijoy, Akata, and SLA) and T cell lines (including Jurkat, H-9, Rex, H-SB2, and Hut-78). However, HB15 expression was generally low and variable. The highest levels of cell-surface expression were always obtained where the cell cultures were recently split and were thus proliferating maximally. Cell lines that did not express detectable levels of HB-15 included: K562; the B cell lines NALM-6 and Ramos; the T cell lines, MOLT-3, RPMI 8405, PEER, MOLT-14, CEM and HPB-ALL; the myelomonocytic line, HL60; the natural killer cell line, YT; the colon carcinoma lines, Colo-205 and HT29; the lung cell lines, NCI-H69, and NCI-H82, the prostate line, PC3; the melanoma line, MEWO; and the breast tumor lines, ZRT5.1, MCF7 and BT20.

Expression of HB15 by normal blood leukocytes was also examined. However, cell-surface expression of HB15 was not detected at significant levels on circulating lymphocytes, natural killer cells or monocytes in 15 blood samples. Therefore, the possibility that HB15 was expressed following cellular activation was examined by inducing T lymphocyte proliferation with the mitogens concanavalin A (ConA), pokeweed mitogen, phytohemag-glutinin-P or phorbol esters (PMA). Expression of HB15 was examined 2, 8, 12, 24, 48, 72,

120 and 240 hours following the initiation of cultures. Appearance of HB15 expression paralleled cellular proliferation such that optimal expression was on days 3 through 5 following the initiation of cultures. Also, the quantity of HB15 expression induced was not correlated with any specific mitogen, but correlated more with the strength of the mitogenic signal such that cell-surface expression was predominantly found on the larger blast cells. Therefore, HB15 was expressed by lymphocytes following activation.

Immunohistological analysis of HB15 expression.

The lymphocyte specificity and tissue distribution of HB15 was also examined by immunohistological analysis of different human tissues. Basically, the anti-HB15a mAb was used to stain thymus, tonsil, spleen, lymph node, kidney, renal pelvis and ureter, Fallopian tube, liver, pancreas, stomach, breast, lung, esophagus, skeletal muscle, skin, uterus, salivary gland, thyroid gland, adrenal gland, heart, appendix and colon. (Referring to Figs. 5A-5F), in most cases, HB15 expression appeared lymphocyte specific in that no significant reactivity was observed in non-lymphoid tissues. Among tonsil and lymph nodes (Fig. 5A), HB15 was expressed reasonably strongly by scattered cells in intrafollicular regions (T cell zones) (Fig. 5C). Although some of these cells may have been lymphoblasts, most were interdigitating reticulum cells (a subpopulation of dendritic cells) since they appeared larger than resting lymphocytes and expressed the CD1 surface molecule (Fig. 5D). Also, some cells (50-80%) within germinal centers (GC; Figs. 5A and 5B) and follicular mantle zones (FM; Fig. 5A), with the morphology of lymphocytes, were weakly $HB15^+$. Among spleen, the $HB15^+$ cells were predominantly restricted to the white pulp, whereas the red pulp remained largely negative. Again, these large, scattered positive cells in the white pulp are likely to be interdigitating reticulum cells or lymphoblasts. Cortical thymocytes were HB15 negative, while a small

subpopulation of medullary cells, presumably thymocytes, was positive (Fig. 5E). Unlike other non-hematopoietic tissues, analysis of skin revealed that some cells with the characteristic scattered branching morphology of Langerhan's cells (a subpopulation of dendritic cells) expressed HB15 at detectable levels (Fig. 5F). Among all non-hematopoietic tissues, where inflammatory infiltrations were apparent, a few scattered lymphocytes were found to express HB15. It is also likely that circulating dendritic cells are $HB15^+$, but because of their low frequency they were not readily detected. Similarly, it is also likely that the malignant counterparts of dendritic cells express HB15 and that this molecule can be used as a diagnostic marker for malignant cells as the L428 cell line, which is a neoplastic cell line that was derived from Hodgkin's disease and may represent interdigitating reticulum cells (Schaadt et al., Int. J. Cancer 26:723-731 (1980)), is HB15 positive.

Experimental Procedures

Isolation of cDNA clones.

The isolation of cDNA clones by differential hybridization has been described (Tedder et al., Mol. Immunol. 25:1321-1330 (1988)). One clone, pB123, was purified, labeled by nick translation (Rigby et al., J. Mol. Biol. 113:237-251 (1977)) and used to isolate homologous cDNA by again screening the same human tonsil cDNA library in λgt11 (Weis et al., Proc. Natl. Acad. Sci., USA 83:5639-5643 (1986)) as described (Zhou et al., Immunogenetics 35:102-111 (1992)). Positive plaques were isolated, cloned and the cDNA inserts were removed by EcoR I digestion and subcloned into pSP65 (Melton et al., Nucleic Acids Res. 12:7035-7056 (1984)). Restriction maps were generated as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, (1982) and nucleotide sequences were determined using the method of Sanger et al., Proc. Natl. Acad. Sci., USA 74:5463-5467 (1977).

A computer search of nucleotide and protein sequences was conducted using the Protein Identification Resource Data (GenBank release 66 and Swiss-Prot-16). Gap penalties of -1 were assessed during sequence homology analysis for each nucleotide or amino acid in the sequence where a gap or deletion occurred.

RNA blot analysis.

Poly(A)$^+$ RNA was isolated as described (Maniatis et al., Molecular Cloning: A Laboratory Manual, (1982)). For Northern-blot analysis, 2 μg of poly(A)$^+$ RNA was denatured with glyoxal, fractionated by electrophoresis through a 1.1% agarose gel and transferred to nitrocellulose (Thomas, Methods Enzymol. 100:255 (1983)). The pB123 cDNA insert used as probe was isolated, nick-translated (Rigby et al., J. Mol. Biol. 113:237-251 (1977)) and hybridized with the filters as described (Wahl et al., Proc. Natl. Acad. Sci., USA 76:3683-3687 (1979)). Hybridization at high stringency was with 50% (v/v) formamide, 4X SSC, 10% (w/v) Na dextran sulfate at 42°C. The filters were washed at 65°C with 0.2X SSC, 0.1% SDS. RNA size was determined by comparison with 28S and 18S ribosomal RNA run on the same gels as standards. The same blot was also hybridized with cDNA clones containing a housekeeping mRNA of unknown identity revealing that all mRNA were intact and were similar in quantity of this expressed mRNA. For hybridization at low stringency the conditions are overnight incubation at 42°C in a solution comprising: 20% formamide, 5XSSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5X Denhardts solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA.

Cells.

Human blood was obtained by protocols approved by the Human Protection Committee of Dana-Farber Cancer Institute and mononuclear cells were isolated by Ficoll-Hypaque density

gradient centrifugation. Mononuclear cells (10^6/ml) in complete media (RPMI-1640 supplemented with 15% fetal calf serum, antibiotics and glutamine) were stimulated with phytohemagglutinin-P (2 μg/ml; Difco, Detroit, MI), Con A (10 μg/ml, Miles Laboratories, Elkhart, IN), pokeweed mitogen (10 μg/ml, Gibco/BRL, Bethesda, MD) or phorbol myristate 13-acetate (PMA, 10 ng/ml, Sigma, St. Louis, MO) as described (Tedder et al., J. Immunol. 144:532-540 (1990)). Lymphocytes were harvested at the indicated time points, washed once in complete media, and aliquoted for immediate immunofluorescence staining as described below.

COS cells were transfected with the pHB15 cDNA insert subcloned into a modified CDM8 vector (Aruffo et al., EMBO J. 6:3313 (1987); Tedder et al., J. Immunol. 143:712-717 (1989)) using the DEAE-dextran method as described (Aruffo et al., EMBO J. 6:3313 (1987)). Cell surface expression was examined after 48 hours by indirect immunofluorescence. Stable cDNA transfected cells were produced using the pHB15 cDNA cloned into the BamH I site of the retroviral vector pZipNeoSV(X) in the correct orientation (Cepko et al., Cell 37:1053-1062 (1984)). The murine pre-B cell line, 300.19, and the human erythroleukemia cell line, K562, were transfected with this vector by electroporation with subsequent selection of stable transfectants using G418 (Gibco/BRL). Cells expressing HB15 were further enriched by reacting the cells with monoclonal antibodies with the subsequent isolation of $HB15^+$ cells by panning on anti-mouse Ig coated plates.

Cell lines were grown in RPMI 1640 medium containing 10% fetal calf serum and antibiotics. Cultures of all cell lines were split the day before analysis and were in logarithmic growth.

mAb production.

Anti-HB15 mAb were generated as described (Tedder et al., J. Immunol. 144:532-540 (1990)) by the fusion of NS-1

myeloma cells with spleen cells from BALB/c mice that were repeatedly immunized with COS cells transfected with the HB15 cDNA. Each hybridoma was cloned twice and used to generate ascites fluid. The isotypes of the mAb were determined using a mouse monoclonal antibody isotyping kit from Amersham (Arlington Heights, IL).

Immunofluorescence analysis.

Cells were kept at 4°C and were examined immediately after isolation. Indirect immunofluorescence analysis of viable cells was carried out after washing the cells three times. The cells were then incubated for 20 min on ice with each mAb as ascites fluid diluted to the optimal concentration for immunostaining. Isotype-matched murine antibodies that were unreactive with human leukocytes were used as negative controls. After washing, the cells were treated for 20 min at 4°C with fluorescein isothiocyanate-conjugated goat anti-mouse Ig antibodies (Southern Biotechnology Associates, Birmingham, AL). Single color immunofluorescence analysis was performed on an Epics Profile flow cytometer (Coulter Electronics, Hialeah, FL). Ten thousand cells were analyzed for each sample.

Immunoprecipitation analysis.

Cells were washed twice, resuspended in saline and labeled by the iodogen method as described (Thompson et al., Biochem. 26:743-750 (1987)). After washing, the cells were lysed in 1 ml of buffer containing 1% (v/v) Triton X-100 and protease inhibitors as described (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)). Immunoprecipitations were carried out using anti-HB15a mAb or mouse Ig (as a negative control) directly conjugated to Affigel (BioRad, Richmond, VA) at 2 mg of mAb per ml of gel according to the manufacturer's instructions. Cell lysates were precleared twice for 2 hours using 50 μl (50% v/v) of murine Ig coated beads at 4°C. Cell lysates were precleared again overnight.

Half of the precleared lysate was then incubated with 25 μl of anti-HB15a mAb-coated beads or murine Ig-coated beads with constant rotation at 4°C for 18 hours. Immunoprecipitates were washed and analyzed by SDS-PAGE as described (Tedder et al., Proc. Natl. Acad. Sci., USA 85:208 (1988)) with half of the sample run in the presence of 5% 2-mercaptoethanol (reducing conditions). M_r were determined using pre-stained standard molecular weight markers (Gibco/BRL).

Immunohistochemistry.

All tissues were stained applying a modification of the APAAP procedure as described by Cordell et al., J. Histochem. Cytochem. 31:219-229 (1984). Basically, the slides were first incubated with monoclonal antibody followed by an incubation step with rabbit anti-mouse (bridging) antibody. Subsequently, a monoclonal antibody against alkaline phosphatase pre-incubated with alkaline phosphatase was applied. In order to enhance the sensitivity of this procedure, the number of phosphatase molecules on the surface was increased by using one or two layers of bridging antibody and anti-phosphatase antibody. Bound phosphatase molecules were visualized using new fuchsin as a substrate (Cordell et al., J. Histochem. Cytochem. 31:219-229 (1984)).

Use

The HB15 protein or immunospecific fragments thereof, or antibodies or other antagonists to HB15 function, can be used to diagnose or treat a variety of immunological disorders, diseases or syndromes. For such purposes, the soluble external domain would often be employed, typically but not necessarily, polymerized in a multivalent state using, e.g., dextran or polyamino acid carriers or fusion proteins of HB15 fragments and carrier molecules. Liposomes may alternatively be employed as the therapeutic vehicle, in which case the transmembrane domain and preferably at least some of the cytoplasmic domain will also be included.

For example, since Langerhans' cells are the primary immunocompetent cell in the skin, playing a role in the presentation of antigen to T cells and the induction of contact hypersensitivity, and since HB15 is expressed by Langerhans' cells and may be involved in antigen presentation, it is likely to be involved in the pathogenesis of human skin disease such as psoriasis, autoimmune disorders, organ transplant and AIDS.

Therefore, antagonists to HB15 function can provide important therapeutic agents for treatment of these diseases. Similarly, since HB15 may serve as an accessory molecule for lymphocyte activation, the HB15 antigen, fragments or domains thereof, may be used as agonists that would augment an immune response.

More specifically, the dendritic cell is a primary target of the human immunodeficiency virus, the causative agent of AIDS. It has recently been proposed that 80% of AIDS virus *in vivo* is produced by dendritic cells, particularly by Langerhans' cells, circulating dendritic cells and interdigitating reticulum cells (Langhoff et al., Proc. Natl. Acad. Sci. USA 88:7998-8002 (1991)). Also, most infections occur through mucosal surfaces where it is thought that dendritic cells are first infected. Therefore, this reagent provides us with a critical tool for the potential prevention or treatment of AIDS or AIDS related disorders.

For monitoring certain clinical conditions, it may be advisable to quantitate the levels of endogenous soluble HB15 in a patient's blood serum. Based on the finding that several receptors are now known to be shed during various normal and pathological conditions, it is possible that HB15 is also lost from the cell surface by an enzymatic process. Also, quantitative detection can be useful in a method of identifying leukocytes with abnormal or decreased expression of HB15 for diagnosis and/or detection of leukocyte activation or altered leukocyte function. Additionally, the ability to quantitate the amount of receptor, or fragment

thereof, produced during the manufacture of a recombinant therapeutic agent will be advantageous. Quantitation of HB15 levels can be carried out using a number of assay methods known to those of ordinary skill in the art, including an enzyme-linked immunoassay using the monoclonal antibodies that have been produced against HB15.

Similarly, in treating certain clinical conditions, it may be advisable to remove endogenous soluble HB15 or $HB15^+$ cells from a patient's blood. This can be done with existing on-line and off-line techniques by employing immunoselection columns containing antibodies or other binding agents directed against the disclosed external domain of HB15.

There are at present no specific markers for non-follicular dendritic cells in humans. Use of HB15 monoclonal antibody to identify $HB15^+$ cells now permits the isolation and purification of cells expressing this protein from a population of unrelated cells.

The HB15 mAb will also be useful for the evaluation and diagnosis of interdigitating cell sarcomas or other malignant cell types expressing this antigen. Therefore, HB15-based agents may be suitable for immunotherapy or immunoimaging.

In addition, assays for HB15 function can be used in further research on the physiological role of this receptor. For example, in preliminary experiments T cell proliferation in the mixed lymphocyte reaction, an assay for T cell activation, can be partially inhibited by the presence of anti-HB15 monoclonal antibodies. This functional assay suggests a role for the HB15 molecule on dendritic cells or monocytes in the initiation of T cell function.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the

definitions contained in the appended claims and equivalents thereof.

Deposits

The following hybridomas were deposited on March 17, 1992, with the American Type Culture Collection (ATCC).

Identification	ATCC Designation
Anti-HB15a Hybridoma cell line, HB15a	HB 10987
Anti-HB15b Hybridoma cell line, HB15b	HB 10988

Applicants' assignee, Dana-Farber Cancer Institute, Inc., represents that the ATCC is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period is longer. Applicants' assignee acknowledges its duty to replace the deposit should the depository be unable to furnish a sample when requested due to the condition of the deposit.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Dana-Farber Cancer Institute, Inc.

(ii) TITLE OF INVENTION: LYMPHOCYTE ACTIVATION ANTIGEN HB15, A MEMBER OF THE IMMUNOGLOBULIN SUPERFAMILY

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Weingarten, Schurgin, Gagnebin & Hayes

(B) STREET: Ten Post Office Square

(C) CITY: Boston

(D) STATE: MA

(E) COUNTRY: USA

(F) ZIP: 02109

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/870,029

(B) FILING DATE: 17-APR-1992

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Heine, Holliday C.

(B) REGISTRATION NUMBER: 34,346

(C) REFERENCE/DOCKET NUMBER: DFCI-230Xq999

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-2290

(B) TELEFAX: (617) 451-0313

(C) TELEX: 940675

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1762 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 11..625

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 68..622

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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           Met Ser Arg Gly Leu Gln Leu Leu Leu Ser Cys Ala
           -19             -15                 -10

TAC AGC CTG GCT CCC GCG ACG CCG GAG GTG AAG GTG GCT TGC TCC GAA    97
Tyr Ser Leu Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu
 -5                  1               5                  10

GAT GTG GAC TTG CCC TGC ACC GCC CCC TGG GAT CCG CAG GTT CCC TAC   145
Asp Val Asp Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr
                15                  20                  25

ACG GTC TCC TGG GTC AAG TTA TTG GAG GGT GGT GAA GAG AGG ATG GAG   193
Thr Val Ser Trp Val Lys Leu Leu Glu Gly Gly Glu Glu Arg Met Glu
            30                  35                  40

ACA CCC CAG GAA GAC CAC CTC AGG GGA CAG CAC TAT CAT CAG AAG GGG   241
Thr Pro Gln Glu Asp His Leu Arg Gly Gln His Tyr His Gln Lys Gly
        45                  50                  55

CAA AAT GGT TCT TTC GAC GCC CCC AAT GAA AGG CCC TAT TCC CTG AAG   289
Gln Asn Gly Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys
    60                  65                  70

ATC CGA AAC ACT ACC AGC TGC AAC TCG GGG ACA TAC AGG TGC ACT CTG   337
Ile Arg Asn Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu
75                  80                  85                  90

CAG GAC CCG GAT GGG CAG AGA AAC CTA AGT GGC AAG GTG ATC TTG AGA   385
Gln Asp Pro Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg
                95                  100                 105

GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT TTT AAG AAA TAC   433
Val Thr Gly Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr
            110                 115                 120

AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA   481
Arg Ala Glu Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr
        125                 130                 135

CTC ATC ATT TTC ACT TGT AAG TTT GCA CGG CTA CAG AGT ATC TTC CCA   529
Leu Ile Ile Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro
    140                 145                 150
```

GAT TTT TCT AAA GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC 577
Asp Phe Ser Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser
155 160 165 170

CCA AAT AAG CAT TTA GGG CTA GTG ACT CCT CAC AAG ACA GAA CTG GTA 625
Pro Asn Lys His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val
175 180 185

TGAGCAGGAT TTCTGCAGGT TCTTCTTCCT GAAGCTGAGG CTCAGGGGTG TGCCTGTCTG 685

TTACACTGGA GGAGAGAAGA ATGAGCCTAC GCTGAAGATG GCATCCTGTG AAGTCCTTCA 745

CCTCACTGAA AACATCTGGA AGGGGATCCC ACCCCATTTT CTGTGGGCAG GCCTCGAAAA 805

CCATCACATG ACCACATAGC ATGAGGCCAC TGCTGCTTCT CCATGGCCAC CTTTTCAGCG 865

ATGTATGCAG CTATCTGGTC AACCTCCTGG ACATTTTTTC AGTCATATAA AAGCTATGGT 925

GAGATGCAGC TGGAAAAGGG TCTTGGGAAA TATGAATGCC CCCAGCTGGC CCGTGACAGA 985

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TTGCTGTACC AGCCCAGATG TTTTACGTCT GGGAGAAATT GACAGATCAA GCTGTGAGAC 1105

AGTGGGAAAT ATTTAGCAAA TAATTTCCTG GTGTGAAGGT CCTGCTATTA CTAAGGAGTA 1165

ATCTGTGTAC AAAGAAATAA CAAGTCGATG AACTATTCCC CAGCAGGGTC TTTTCATCTG 1225

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GTACTTGTCA AAGAAGGTTT GTGTTTTTCT GCTTTTGAAA TCTGTATCTG TAGTGAGATA 1345

GCATTGTGAA CTGACAGGCA GCCTGGACAT AGAGAGGGAG AAGAAGTCAG AGAGGGTGAC 1405

AAGATAGAGA GCTATTTAAT GGCCGGCTGG AAATGCTGGG CTGACGGTGC AGTCTGGGTG 1465

CTCGTCCACT TGTCCCACTA TCTGGGTGCA TGATCTTGAG CAAGTTCCTT CTGGTGTCTG 1525

CTTTCTCCAT TGTAAACCAC AAGGCTGTTG CATGGGCTAA TGAAGATCAT ATACGTGAAA 1585

ATTCTTTGAA AACATATAAA GCACTATACA GATTCGAAAC TCCATTGAGT CATTATCCTT 1645

GCTATGATGA TGGTGTTTTG GGGATGAGAG GGTGCTATCC ATTTCTCATG TTTTCCATTG 1705

TTTGAAACAA AGAAGGTTAC CAAGAAGCCT TTCCTGTAGC CTTCTGTAGG AATTCCA 1762

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 205 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Arg Gly Leu Gln Leu Leu Leu Ser Cys Ala Tyr Ser Leu
-19 -15 -10 -5

Ala Pro Ala Thr Pro Glu Val Lys Val Ala Cys Ser Glu Asp Val Asp
1 5 10

Leu Pro Cys Thr Ala Pro Trp Asp Pro Gln Val Pro Tyr Thr Val Ser
15 20 25

Trp Val Lys Leu Leu Glu Gly Gly Glu Glu Arg Met Glu Thr Pro Gln

30 35 40 45

Glu Asp His Leu Arg Gly Gln His Tyr His Gln Lys Gly Gln Asn Gly
50 55 60

Ser Phe Asp Ala Pro Asn Glu Arg Pro Tyr Ser Leu Lys Ile Arg Asn
65 70 75

Thr Thr Ser Cys Asn Ser Gly Thr Tyr Arg Cys Thr Leu Gln Asp Pro
80 85 90

Asp Gly Gln Arg Asn Leu Ser Gly Lys Val Ile Leu Arg Val Thr Gly
95 100 105

Cys Pro Ala Gln Arg Lys Glu Glu Thr Phe Lys Lys Tyr Arg Ala Glu
110 115 120 125

Ile Val Leu Leu Leu Ala Leu Val Ile Phe Tyr Leu Thr Leu Ile Ile
130 135 140

Phe Thr Cys Lys Phe Ala Arg Leu Gln Ser Ile Phe Pro Asp Phe Ser
145 150 155

Lys Ala Gly Met Glu Arg Ala Phe Leu Pro Val Thr Ser Pro Asn Lys
160 165 170

His Leu Gly Leu Val Thr Pro His Lys Thr Glu Leu Val
175 180 185

CLAIMS

What is claimed is:

1. A nucleic acid isolate able to hybridize under stringent conditions to the complement of a sequence encoding the extracellular Ig-like domain, the transmembrane domain, or the cytoplasmic domain of the HB15 protein having the amino acid sequence shown in Fig. 2 and encoding a polypeptide having HB15 biological activity.

2. The nucleic acid isolate of claim 1 wherein said nucleic acid is DNA.

3. The nucleic acid isolate of claim 1 wherein said nucleic acid is RNA.

4. The nucleic acid isolate of claim 1 wherein the nucleic acid encodes a polypeptide having the amino acid sequence shown in Fig. 2.

5. The nucleic acid isolate of claim 1 wherein the nucleic acid encodes a polypeptide that is recognized by a monoclonal antibody specific for an HB15 determinant.

6. The nucleic acid isolate of claim 1, comprising nucleic acid encoding an HB15 extracellular Ig-like domain and a transmembrane domain.

7. The nucleic acid isolate of claim 1, comprising nucleic acid encoding an HB15 extracellular Ig-like domain free of transmembrane domains and cytoplasmic domains.

8. The nucleic acid isolate of claim 1, comprising nucleic acid encoding an HB15 transmembrane domain free of extracellular Ig-like domains and cytoplasmic domains.

9. The nucleic acid isolate of claim 1, comprising nucleic acid encoding an HB15 cytoplasmic domain free of extracellular Ig-like domains and transmembrane domains.

10. The nucleic acid isolate of claim 1, having nucleic acid encoding an HB15 extracellular Ig-like domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the nucleic acid encoding the HB15 cytoplasmic domain is replaced by a heterologous cytoplasmic domain.

11. The nucleic acid isolate of claim 1, having nucleic acid encoding an HB15 extracellular Ig-like domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the nucleic acid encoding the HB15 transmembrane domain is replaced by a heterologous transmembrane domain.

12. The nucleic acid isolate of claim 1, having nucleic acid encoding an HB15 extracellular Ig-like domain, an HB15 transmembrane domain, and an HB15 cytoplasmic domain, wherein the nucleic acid encoding the HB15 transmembrane domain is replaced by a heterologous transmembrane domain.

13. A recombinant expression vector comprising a nucleic acid sequence able to hybridize under stringent conditions to the complement of a sequence encoding the extracellular Ig-like domain, the transmembrane domain, or the cytoplasmic domain of the HB15 protein having the amino acid sequence shown in Fig. 2 and encoding a polypeptide having HB15 biological activity.

14. A composition comprising a cell transformed with the recombinant expression vector of claim 13.

15. A method of producing the HB15 protein that comprises transforming a host cell with a nucleic acid able to hybridize under stringent conditions to the complement of a

sequence encoding the extracellular Ig-like domain, the transmembrane domain, or the cytoplasmic domain of the HB15 protein having the amino acid sequence shown in Fig. 2 and encoding a polypeptide having HB15 biological activity, culturing the transformed cell, and recovering said HB15 protein from the cell culture.

16. A nucleic acid sequence greater than about 10 bp, capable of hybridizing under stringent conditions to the complement of the nucleic acid sequence shown in Fig. 2.

17. The nucleic acid sequence of claim 16 wherein said nucleic acid is DNA.

18. The nucleic acid sequence of claim 16 wherein said nucleic acid is RNA.

19. The nucleic acid sequence of claim 16 wherein the stringent conditions are overnight incubation at 42°C in a solution comprising: 20% formamide, 5XSSC (150 mM NaCl, 15 mM trisodium citrate); 50 mM sodium phosphate (pH 7.6), 5X Denhardts solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA.

20. The nucleic acid sequence of claim 16 capable of binding to a nucleic acid sequence encoding the HB15 protein having the amino acid sequence shown in Fig. 2 to block transcription of said nucleic acid encoding said HB15 protein.

21. The nucleic acid sequence of claim 16, said sequence being a genomic sequence.

22. The nucleic acid sequence of claim 16 wherein said sequence is a biologically active fragment.

23. The nucleic acid sequence of claim 22 wherein said fragment is from the coding region of the HB15 protein.

24. The nucleic acid sequence of claim 16 ligated to nucleic acid encoding a carrier protein.

25. The nucleic acid sequence of claim 24 wherein said ligated nucleic acid encoding a carrier protein is from a non-human source.

26. The nucleic acid sequence of claim 16 wherein said sequence comprises a fragment of the nucleic acid sequence of Fig. 2 that is greater than about 10 bp.

27. The nucleic acid sequence of claim 16 wherein said sequence comprises a fragment of the nucleic acid sequence of Fig. 2 that is greater than about 20 bp.

28. The nucleic acid sequence of claim 16 wherein said sequence comprises a fragment of the nucleic acid sequence of Fig. 2 that is greater than about 50 bp.

29. The nucleic acid sequence of claim 16 wherein said sequence comprises a fragment of the nucleic acid sequence of Fig. 2 that is greater than about 100 bp.

30. The nucleic acid sequence of claim 16 wherein said sequence comprises an HB15 extracellular Ig-like domain.

31. Cells transfected with at least one strand of the nucleic acid molecule of claim 16, operably linked to suitable control sequences.

32. Cells that do not express the nucleic acid of claim 16 that are transfected with at least one strand of the nucleic

acid of claim 16, operably linked to suitable control sequences.

33. A method of producing a polypeptide encoded by the nucleic acid of claim 16 comprising culturing the cells of claim 32 under conditions effective for the production of said polypeptide and recovering said polypeptide.

34. A polypeptide that is encoded by the nucleic acid isolate of claim 1.

35. A polypeptide having the sequence of the polypeptide of claim 34.

36. A polypeptide that is encoded by the nucleic acid isolate of claim 16.

37. A polypeptide having the sequence of the polypeptide of claim 36.

38. The polypeptide of claim 35 or 37 joined to a carrier molecule.

39. A method of isolating a nucleic acid sequence that cross-hybridizes with the nucleic acid isolate of claim 1 comprising the steps of

providing the nucleic acid isolate of claim 1;

providing a population of nucleic acid molecules;

hybridizing said isolate with said population; and

isolating nucleic acid to which said isolate cross-hybridizes.

40. The method of claim 39 further comprising the step of cloning said nucleic acid to which said isolate cross-hybridizes.

41. The method of claim 40 further comprising the steps of comparing the sequence of said cloned nucleic acid to the sequence of said nucleic acid isolate and continuing to isolate and clone additional cloned nucleic acid sequences and to compare the sequences of said additional cloned nucleic acids to the sequence of said isolate.

42. A method of isolating nucleic acid homologous to the nucleic acid of claim 4 and from an animal species other than human, said method comprising the steps of

providing the nucleic acid isolate of claim 4;

providing a population of nucleic acid molecules from an animal species other than human;

hybridizing said isolate with said population;

isolating and cloning nucleic acid to which said isolate cross-hybridizes;

comparing the sequence of said cloned nucleic acid to the sequence of said nucleic acid isolate; and

continuing to isolate and clone additional cloned nucleic acid sequences and to compare the sequences of said additional cloned nucleic acids to the sequence of said nucleic acid isolate until the sequences of said additional cloned nucleic acids essentially overlap the sequence of said nucleic acid isolate.

43. The method of claim 42 wherein said isolated nucleic acid is from a population of murine nucleic acids.

44. Nucleic acid isolated by the method of claim 42.

45. Nucleic acid isolated by the method of claim 43.

46. Nucleic acid able to hybridize under stringent conditions to the nucleic acid of claim 44.

47. Nucleic acid able to hybridize under stringent conditions to the nucleic acid of claim 45.

48. A polypeptide encoded by the nucleic acid of claim 44 and having HB15 biological activity.

49. A polypeptide having the sequence of the polypeptide of claim 48.

50. A polypeptide encoded by the nucleic acid of claim 45 and having HB15 biological activity.

51. A polypeptide having the sequence of the polypeptide of claim 50.

52. Antibody reactive with the polypeptide of claim 35 or 37.

53. Antibody reactive with a molecule that specifically associates with the polypeptide of claim 35 or claim 37 to generate a functional molecule.

54. A method of identifying cells expressing HB15 comprising reacting the antibody of claim 52 with a population of cells and isolating cells to which said antibody binds.

55. A method of identifying cells expressing HB15 comprising reacting the antibody of claim 52 with a population of cells and detecting cells to which said antibody binds.

56. An imaging agent for imaging a disease state in a human patient comprising a monoclonal antibody reactive with HB15 or ligand binding fragment of HB15 labeled with a radionuclide, paramagnetic isotope or a radiopaque label.

57. A method of quantitating the amount of HB15 expressed on a population of cells comprising

reacting the antibody of claim 52 with said population of cells;

detecting cells to which said antibody binds; and

quantitating the amount of said antibody bound to said population of cells.

58. A method of blocking HB15 protein function comprising reacting antibody of claim 52 with a population of cells expressing said HB15 protein.

59. A method of treating a human patient suffering from cancer, tissue damage, or an immunological disorder comprising administering to said patient a therapeutic amount of an agonist or an antagonist to HB15 function in a non-toxic pharmaceutical carrier therefor.

60. A method of treating a human patient suffering from cancer, tissue damage, or an immunological disorder comprising administering to said patient a therapeutic amount of a polypeptide of claim 35 or claim 37 in a non-toxic pharmaceutical carrier therefor.

61. The use of the polypeptide of claim 35 or claim 37 to identify a ligand that binds to said polypeptide.

62. The use of the polypeptide of claim 35 or claim 37 to identify a ligand that binds to a molecule specifically associated with said polypeptide to generate a functional molecule.

63. A ligand identified using the procedure of claim 61.

64. A ligand identified using the procedure of claim 62.

65. The method of claim 59 wherein said antagonist comprises a ligand, or a portion thereof, identified using the procedure of claim 61 or 62.

66. The method of claim 59 wherein said antagonist comprises the antibody of claim 52 or 53.

67. The method of claim 59 wherein said agonist or antagonist comprises the polypeptide of claim 35 or claim 37.

68. The method of claim 59 wherein said agonist or antagonist comprises the polypeptide of claim 35 or claim 37 joined to a carrier molecule.

69. A human monoclonal antibody reactive with the HB15 protein.

70. The monoclonal antibody of claim 69 wherein said monoclonal antibody is a chimerized monoclonal antibody having variable region segments derived from a mouse monoclonal antibody and other regions derived from a human antibody.

71. The monoclonal antibody anti-HB15a.

72. A monoclonal antibody that recognizes the HB15 epitope(s) recognized by the anti-HB15a monoclonal antibody.

73. The hybridoma cell line deposited as ATCC No. HB 10987.

74. The monoclonal antibody anti-HB15b.

75. A monoclonal antibody that recognizes the HB15 epitope(s) recognized by the anti-HB15b monoclonal antibody.

76. The hybridoma cell line deposited as ATCC No. HB 10988.

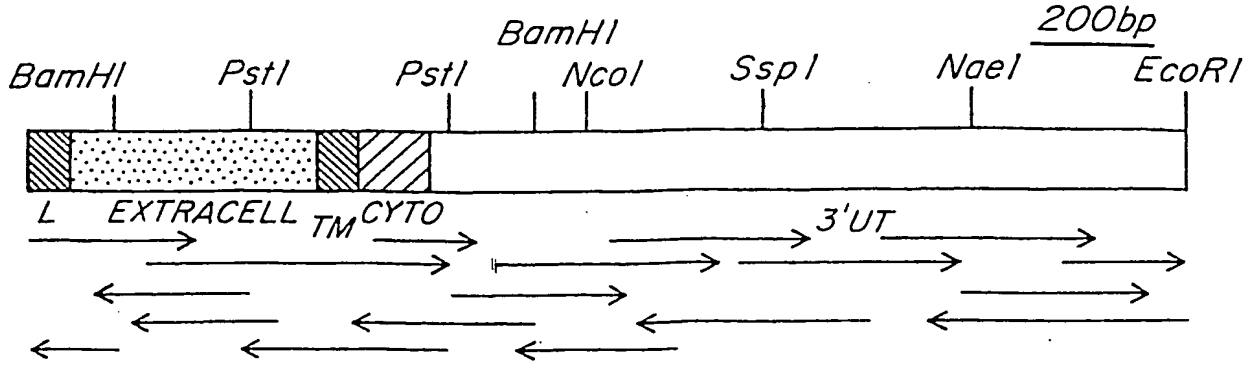
BamHI
PstI
PstI
BamHI
NcoI
SspI
NaeI
EcoRI
200bp
L
EXTRACELL
TM
CYTO
3'UT

FIG. 1

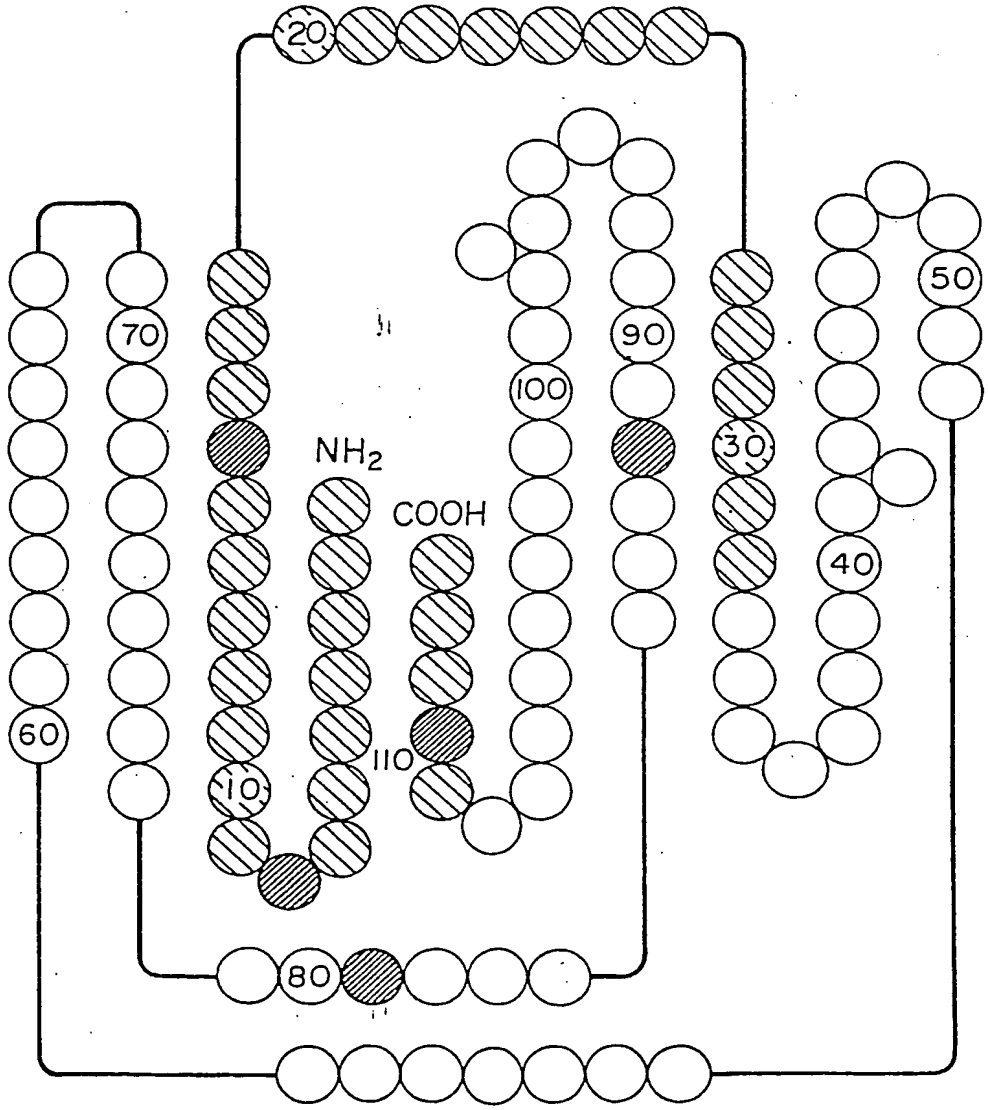
20
70
90
50
100
30
NH2
COOH
40
60
110
10
80

FIG. 3

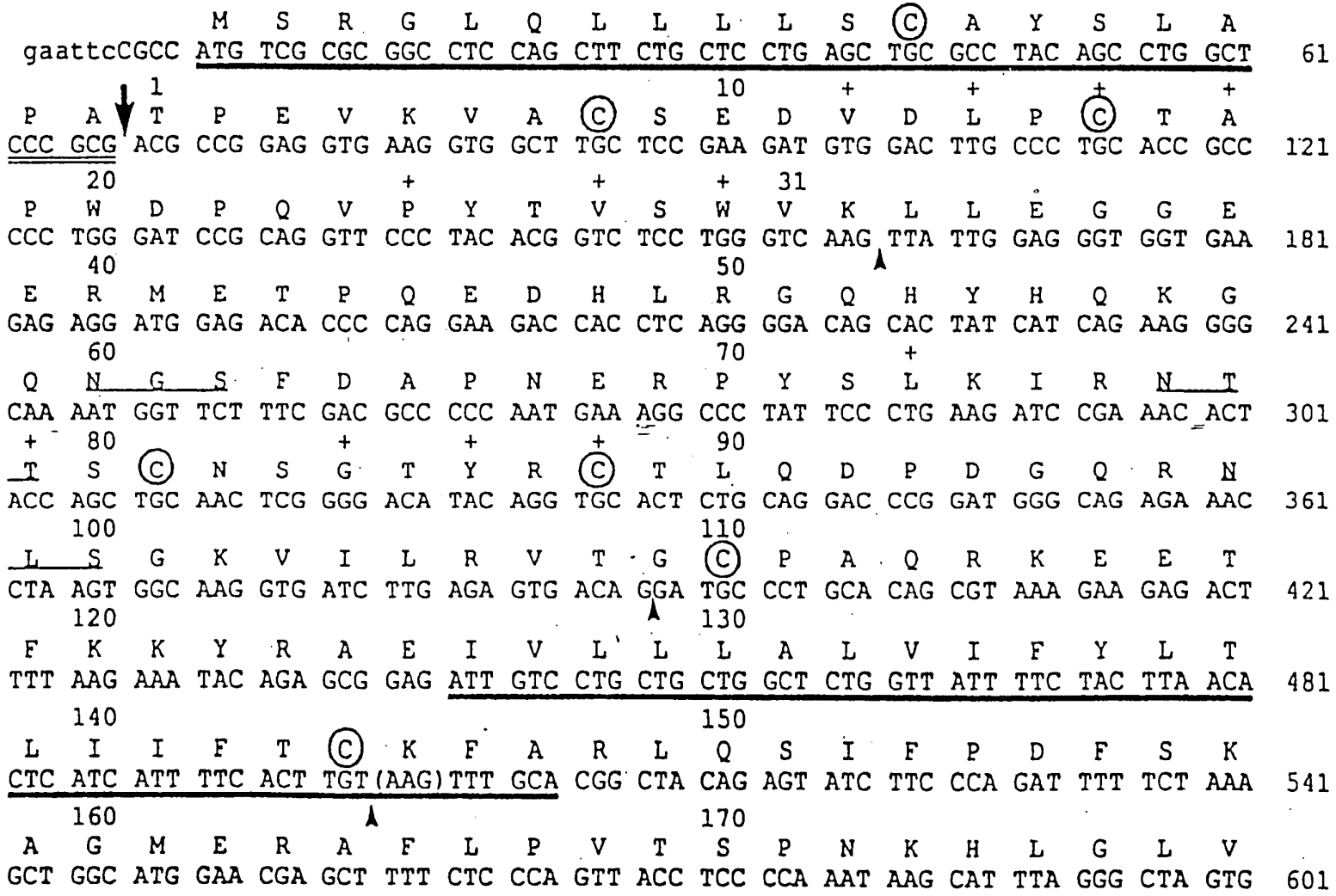
M S R G L Q L L L L S C A Y S L A
gaattcCGCC ATG TCG CGC GGC CTC CAG CTT CTG CTC CTG AGC TGC GCC TAC AGC CTG GCT 61
1 10 + + + +
P A T P E V K V A C S E D V D L P C T A
CCC GCG ACG CCG GAG GTG AAG GTG GCT TGC TCC GAA GAT GTG GAC TTG CCC TGC ACC GCC 121
20 + + + 31
P W D P Q V P Y T V S W V K L L E G G E
CCC TGG GAT CCG CAG GTT CCC TAC ACG GTC TCC TGG GTC AAG TTA TTG GAG GGT GGT GAA 181
40 50
E R M E T P Q E D H L R G Q H Y H Q K G
GAG AGG ATG GAG ACA CCC CAG GAA GAC CAC CTC AGG GGA CAG CAC TAT CAT CAG AAG GGG 241
60 70 +
Q N G S F D A P N E R P Y S L K I R N T
CAA AAT GGT TCT TTC GAC GCC CCC AAT GAA AGG CCC TAT TCC CTG AAG ATC CGA AAC ACT 301
+ 80 + + + 90
T S C N S G T Y R C T L Q D P D G Q R N
ACC AGC TGC AAC TCG GGG ACA TAC AGG TGC ACT CTG CAG GAC CCG GAT GGG CAG AGA AAC 361
100 110
L S G K V I L R V T G C P A Q R K E E T
CTA AGT GGC AAG GTG ATC TTG AGA GTG ACA GGA TGC CCT GCA CAG CGT AAA GAA GAG ACT 421
120 130
F K K Y R A E I V L L L A L V I F Y L T
TTT AAG AAA TAC AGA GCG GAG ATT GTC CTG CTG CTG GCT CTG GTT ATT TTC TAC TTA ACA 481
140 150
L I I F T C K F A R L Q S I F P D F S K
CTC ATC ATT TTC ACT TGT (AAG) TTT GCA CGG CTA CAG AGT ATC TTC CCA GAT TTT TCT AAA 541
160 170
A G M E R A F L P V T S P N K H L G L V
GCT GGC ATG GAA CGA GCT TTT CTC CCA GTT ACC TCC CCA AAT AAG CAT TTA GGG CTA GTG 601

FIG. 2A

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 180                             186
  T   P   H   K   T   E   L   V   *
ACT CCT CAC AAG ACA GAA CTG GTA TGA GCAGGATTTC TGCAGGTTCT TCTTCCTGAA GCTGAGGCTC  668
 AGGGGTGTGC CTGTCTGTTA CACTGGAGGA GAGAAGAATG AGCCTACGCT GAAGATGGCA TCCTGTGAAG  738
 TCCTTCACCT CACTGAAAAC ATCTGGAAGG GGATCCCACC CCATTTTCTG TGGGCAGGCC TCGAAAACCA  808
 TCACATGACC ACATAGCATG AGGCCACTGC TGCTTCTCCA TGGCCACCTT TTCAGCGATG TATGCAGCTA  878
 TCTGGTCAAC CTCCTGGACA TTTTTTCAGT CATATAAAAG CTATGGTGAG ATGCAGCTGG AAAAGGGTCT  948
 TGGGAAATAT GAATGCCCCC AGCTGGCCCG TGACAGACTC CTGAGGACAG CTGTCCTCTT CTGCATCTTG 1018
 GGGACATCTC TTTGAATTTT CTGTGTTTTG CTGTACCAGC CCAGATGTTT TACGTCTGGG AGAAATTGAC 1088
 AGATCAAGCT GTGAGACAGT GGGAAATATT TAGCAAATAA TTTCCTGGTG TGAAGGTCCT GCTATTACTA 1158
 AGGAGTAATC TGTGTACAAA GAAATAACAA GTCGATGAAC TATTCCCCAG CAGGGTCTTT TCATCTGGGA 1228
 AAGACATCCA TAAAGAAGCA ATAAAGAAGA GTGCCACATT TATTTTTATA TCTATATGTA CTTGTCAAAG 1298
 AAGGTTTGTG TTTTTCTGCT TTTGAAATCT GTATCTGTAG TGAGATAGCA TTGTGAACTG ACAGGCAGCC 1368
 TGGACATAGA GAGGGAGAAG AAGTCAGAGA GGGTGACAAG ATAGAGAGCT ATTTAATGGC CGGCTGGAAA 1438
 TGCTGGGCTG ACGGTGCAGT CTGGGTGCTC GTCCACTTGT CCCACTATCT GGGTGCATGA TCTTGAGCAA 1508
 GTTCCTTCTG GTGTCTGCTT TCTCCATTGT AAACCACAAG GCTGTTGCAT GGGCTAATGA AGATCATATA 1578
 CGTGAAAATT CTTTGAAAAC ATATAAAGCA CTATACAGAT TCGAAACTCC ATTGAGTCAT TATCCTTGCT 1648
 ATGATGATGG TGTTTTGGGG ATGAGAGGGT GCTATCCATT TCTCATGTTT TCCATTGTTT GAAACAAAGA 1718
 AGGTTACCAA GAAGCCTTTC CTGTAGCCTT CTGTAGGAAT TCC                              1761
```

FIG. 2B

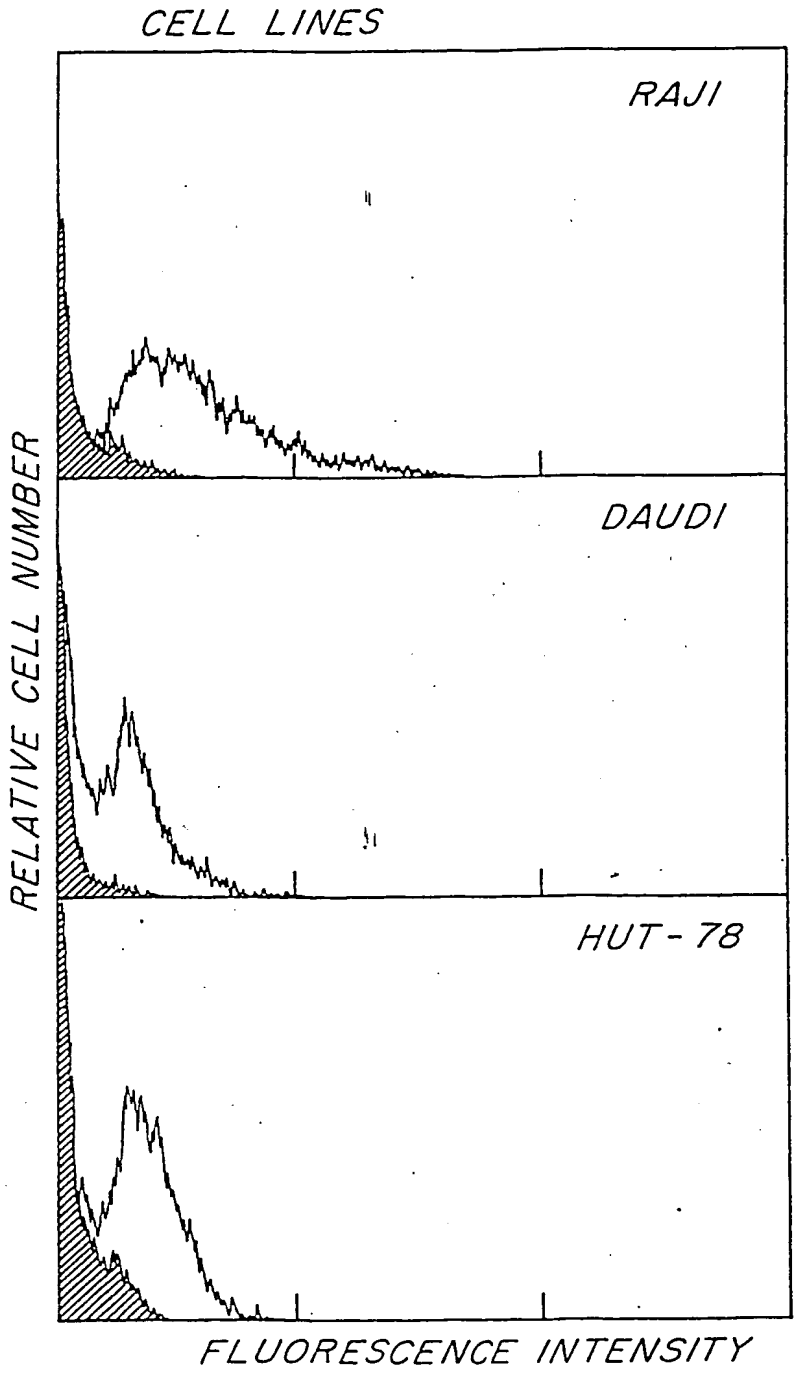
CELL LINES
RAJI
DAUDI
HUT-78
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY

FIG. 4A

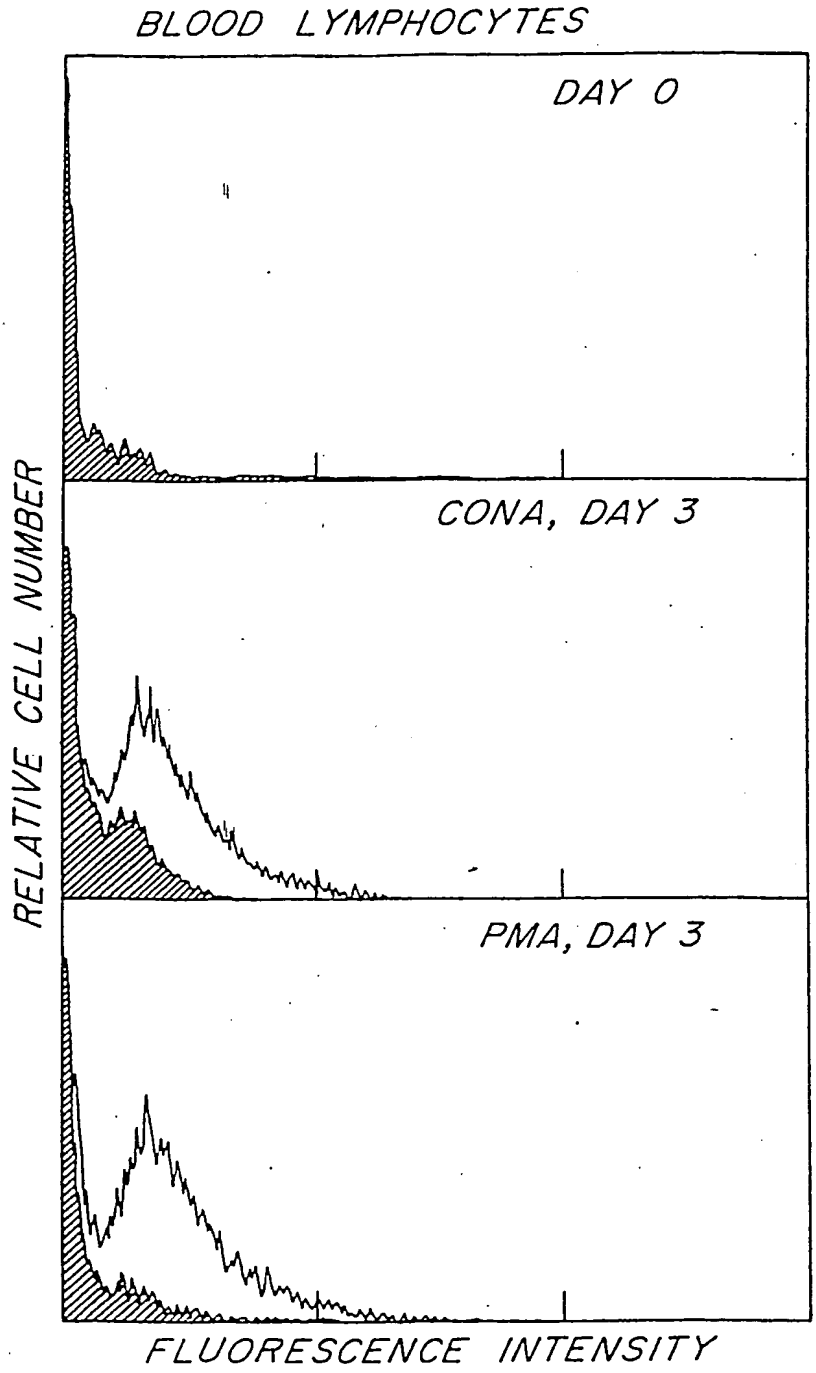
BLOOD LYMPHOCYTES
DAY 0
CONA, DAY 3
PMA, DAY 3
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY

FIG. 4B

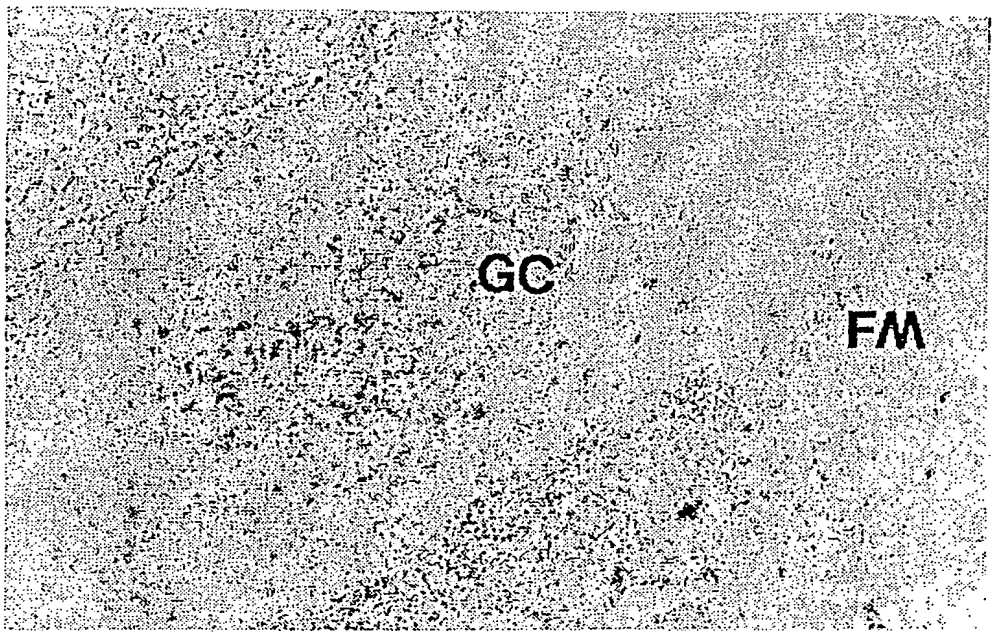

FIG. 5A

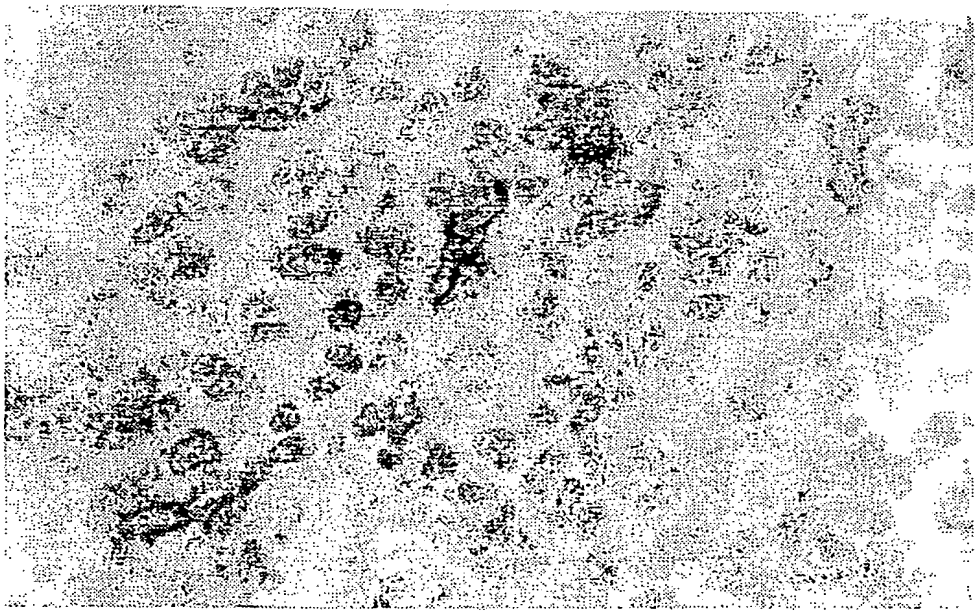

FIG. 5B

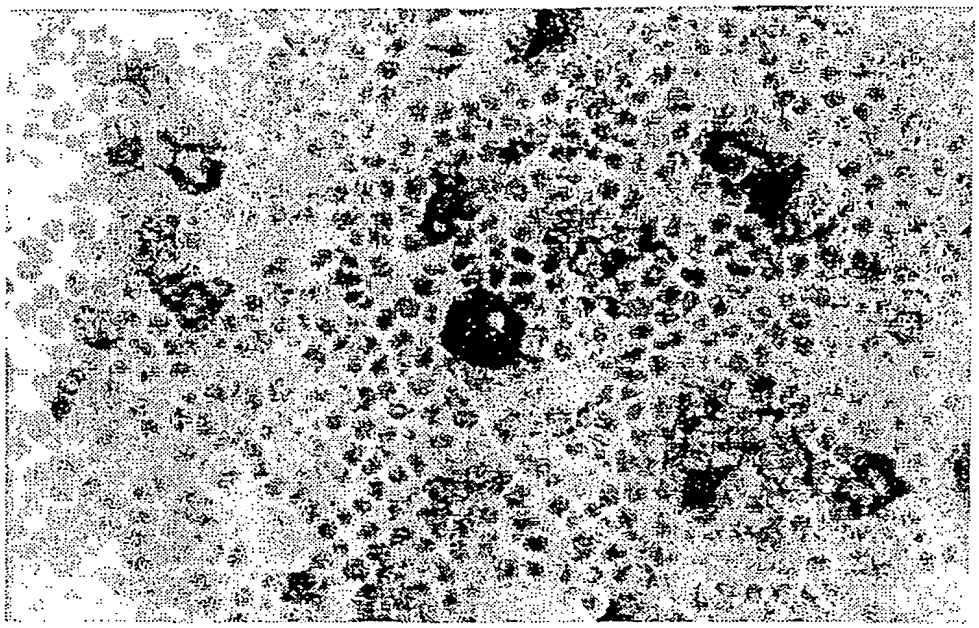

FIG. 5C

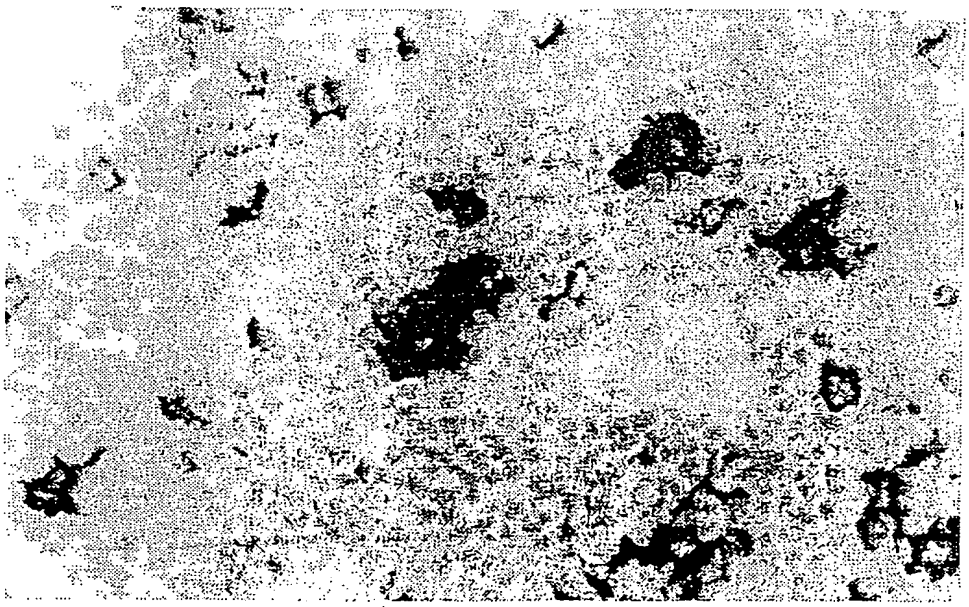

FIG. 5D

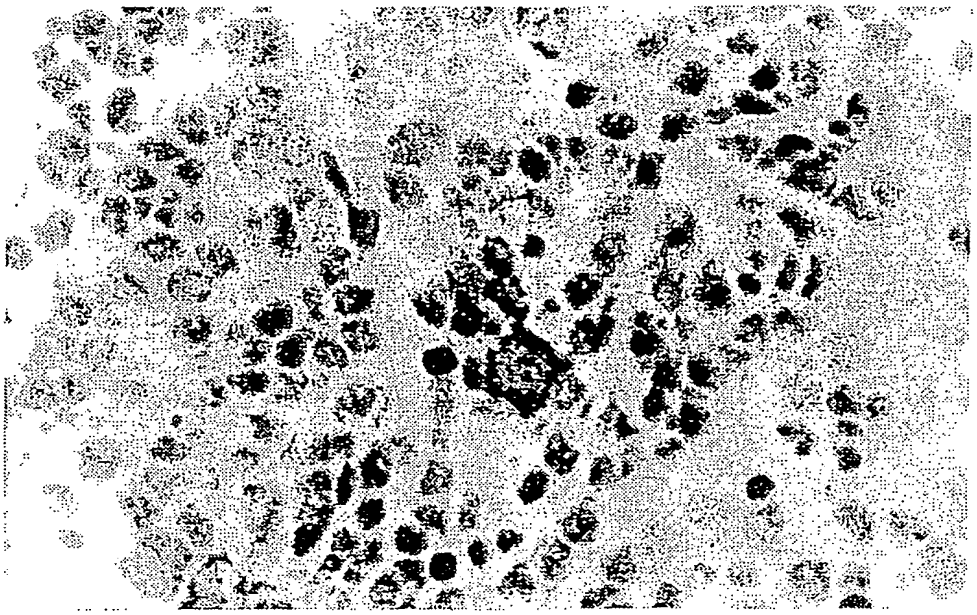

FIG. 5E

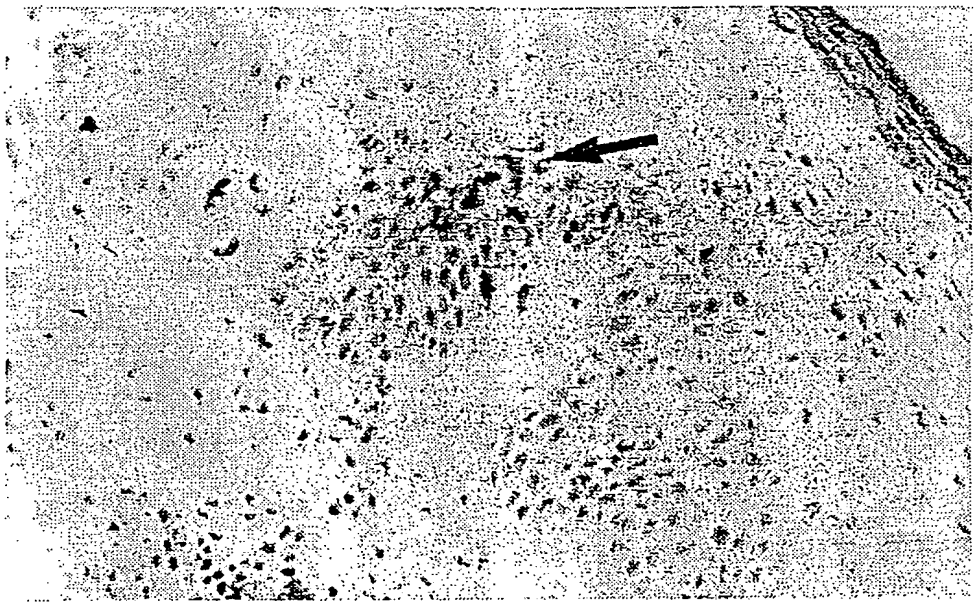

FIG. 5F

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03577

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C07K15/00; C12N5/10; A61K37/02
C12P21/08; G01N33/68; A61K49/02; C12Q1/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EMBL Database entry EBV, accession no. V01555 et al.; 1983; Epstein-Barr virus genome. *sequence* ---	16,17, 19,31,32
A	EP,A,0 386 906 (DANA FARBER CANCER INSTITUTE) 12 September 1990 *column 4, line 40 - column 5, line 40; claims* ---	1
A	WO,A,9 201 049 (THE GENERAL HOSPITAL CORPORATION) 23 January 1992 *page 9, line - page 14, line 4; Rxample XIII; claims* ---	1

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 SEPTEMBER 1993	01 -10- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	YEATS S.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,9 110 682 (INSERM ET AL.) 25 July 1991 *revendications*	1
A	JOURNAL OF IMMUNOLOGY vol. 143, 1989, pages 712 - 717 T.F. TEDDER ET AL.; 'Isolation of cDNAs encoding the CD19 antigen of human and mouse B lymphocytes' cited in the application *abstract*	1
P,X	JOURNAL OF IMMUNOLOGY vol. 149, 1992, pages 735 - 742 L.-J. ZHOU ET AL.; 'A novel cell-surface molecule expressed by human interdigitating reticulum cells, Langerhans cells, and activated lymphocytes is a new member of the Ig superfamily' *whole document*	1-76

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9303577
SA 74051

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 22/09/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0386906	12-09-90	CA-A-	2010321	21-08-90
		JP-A-	3022982	31-01-91
WO-A-9201049	23-01-92	AU-A-	8528691	04-02-92
		EP-A-	0551301	21-07-93
WO-A-9110682	25-07-91	FR-A-	2656800	12-07-91
		EP-A-	0510079	28-10-92
		JP-T-	5503520	10-06-93